Please replace Claims 1, 2, 7-10, 13 and 16 with the attached clean version of the replacement claims.

Please see a marked up version of the amended claims attached hereto to aid the Examiner in identification of the changes.

REMARKS

Claims 1-16 are presented for examination.

The claims were rejected under 35 USC §112, first paragraph, for not describing in the specification in a way to enable one skilled in the art to practice the invention. Specifically, the Examiner states that the claims recite the use of ß-(1,6)-glucanase enzyme to prepare the ß-glucan product. Further, Claims 2 and 3 recite specific microorganisms that the enzyme is attained from. The Examiner believes that the specification does not provide guidance regarding how to obtain the enzyme, nor does it show how the enzyme is obtained from the microorganisms recited in Claims 2 and 3. Therefore, the Examiner believes that one skilled in the art would need to perform undue experimentation to practice the invention.

The present invention describes a series of processes for preparing novel glucan products that have increased efficacy as an immunostimulant as compared to prior art. As described in the specification, beta-glucans from yeast are composed of ß-1,3-glucan chains and beta-1,6-glucan chains. The backbone of the beta-glucan in yeast is composed of ß -1,3-linked chains with branches extending from this backbone. These branches (side chains) are all anchored through a ß-1,6-linkage to the backbone, but the linkage composition within the side chain itself can either be ß-1,6-linked glucosyl units or ß-1,3-linked glucosyl units. There is also a possibility that some intra-chain ß -1,6-linkages could exist also within the backbone. In the present invention, it was surprisingly found that when using an enzyme to remove the ß -1,6-linked chains (i.e., consecutively ß -1,6-linked glucosyl residues) the resulting product had increased immunostimulatory efficacy. This was surprising since a number of prior art publications

from the early 1990's claimed that an engineered and modified yeast strain with increased number of ß -1,6-linked side chains (i.e., where the side chain itself is ß -1,6-linked) was said to be superior as immunostimulant. Reference is made to publications and patents describing the use of PGG (Poly- ß -1,6-glucotriosyl- ß -1,3-glucopyranose glucan) also known as Betafectin. This product was made from an engineered yeast strain, identified as R4, expressing increased number of ß -1,6-linked side chains as evident from the first part of the name of the product "Poly- ß -1,6-glucotriosyl-..." (i.e., three consecutive ß -1,6-linked glucosyl units) and also described in drawings in the publication, "Spectral Analysis of Glucan Produced by Wild-Type and Mutant *Saccharomyces cerevisiae*" Jamas S, Chen Y-CJ, von der Osten CH, Sinskey AJ, Rha CK. Carbohydrate Polymers 13:207-19, 1990. The biological advantage of the product is further described in "Anti-infective effect of poly-ß-1-6-Glucotriosyl-ß-1-3-glucopyranose glucan *in vivo*" Onderdonk AB, Cisneros RL, Hinkson P, Ostroff G. Infection and Immunity 60:1642-7, 1992, and also in many later publications. The R4 yeast strain is also described in the Jamas reference, US Patent No. 5,028,703, wherein it is stated in column 4, lines 12-15, "The strain *Saccharomyces cerevisiae* R4 is a mutant strain of A364A which has been isolated on the basis of an increased proportion of ß-(1,6) linkages". These references and others are being submitted in an Information Disclosure Statement with this Response.

The present invention, in contrast to the above, describes how to modify beta-glucans from yeast by removing ß -1,6-linked chains from the product and thus increase its immunostimulatory activity, by using an endo- ß -1,6-glucanase. It should be stressed that the enzyme treatment does not affect chains having ß -1,3-linkages, whether in the backbone or in the side chains. The ß -1,3-linked side chains are located where the side chain is anchored to the main chain through a ß -1,6-linkage and thus are not affected by the treatment. The resulting product is thus a branched glucan where the side chains are in essence ß -1,3-linked, with the exception of the remnants from chains where the endo-glucanase does not work, those of 4 or less units. This is because of the nature of an endo-enzyme, which requires a certain length of the chain in order to function.

There are other possibilities to alter the amount of ß -1,6-linkages in the glucan. The prior art either describes the advantages of increasing the amount of ß -1,6-linkages as seen in the Jamas US Patent No. 5,028,703, the Jamas '90 article, and the Onderdonk article or the methods are ineffective or even destructive in the way that they do not discriminate between the chains of ß -1,6-glucans and the ß -1,6-branching point where ß -1,3-linked side chains are anchored to the backbone (e.g., hydrochloric acid treatment).

It should be understood that it is unexpected that an endo- ß -1,6-glucanase is able to degrade ß -1,6-linked glucosyl chains, as also described by the Shiota et al. reference. The present invention, however, relates to the fact that using this enzyme to treat ß -glucans from yeast increases their immunostimulatory potential without the need of using chemical treatments that are highly unspecific in their action, and thus possibly resulting in deterioration of the immunostimulatory activity of the resulting product.

None of the foregoing has been described in the prior art. On the contrary, the prior art describes the advantages of having an increased amount of ß -1,6-linkages. The present invention relates to the removal of ß -1,6-glucans, but with maintenance of the branched structure of the glucan with ß -1,3-linked side chains.

Claims 1-16 have been rejected under 35 USC §112, first paragraph, as containing subject matter which is not described in the specification to enable one skilled in the art to make the invention. The Examiner states that the claims recite the use of ß-(1,6) glucanase enzyme to prepare a ß -glucan product. The Examiner further states that Claims 2 and 3 recite specific microorganisms that the enzyme is obtained from. The Examiner further states that the specification provides no guidance regarding how to obtain the enzyme, nor how the enzyme is to obtained from the microorganisms recited in Claims 2 and 3. It is stated by the Examiner that it would take undue experimentation by one skilled in the art to prepare the enzymes required to practice the claimed invention. Applicants strongly disagree with the Examiner's statement. One skilled in the art would know how to obtain the endoenzyme and how to purify it. Methods for isolating endo-glucanases from various sources have been known in the art for many years. Various publications, all of which are dated well before the filing of the

above-identified patent application, which describe methods for isolating endo-glucanases from various sources are enclosed in an Information Disclosure Statement. For example, in an article entitled *Pilot Scale Production of Trichoderma reesei* endo-ß-glucanase by brewer's yeast by Zurbriggen et al., published in the Journal of Bacteriology, 17(1991) 133-146, endo-ß-glucanase I of *Trichoderma reesei* was produced in a laboratory and pilot scale quantities using recombinant strains of *Saccharomyces cereviseae*. In this process, the gene EG/L was integrated in the chromosome or an expression cassette was inserted on a multi-copy plasmid. Expression levels were compared in laboratory scale bioreactor. The best glucanase producing strain was cultivated in a pilot scale. In concentrating the enzyme, absorbent treatment was used to remove endogeneous yeast proteins and other impurities from the culture filtrate. One step purification of the endo-ß-glucanase I protein was obtained using DEAE-sepharose on which the endo-ß-glucanase was bound.

In another example, an article entitled *Purification of Properties of Endo-ß-(1,6) Glucanase* from *Rhizopus chinensis* by Yamamoto et al., AgrA. Biol. Chem., 38(8), 1693-1500, 1974, there is a description of an endo-ß-(1,6) glucanase being purified from the culture filtrate of a strain resembling *Rhizopus chinensis* in homogeneous form. The process involves ammonium sulfate fractionation followed by column chromatography of DEAE-cellulose, CM-Sephadex C-50 and BioGel P-60.

It is clear from the foregoing publications that one skilled in the art would know how to obtain the ß-(1,6) glucanase enzyme used to prepare a beta-glucan product of the present invention well before the filing of the present application. Since one skilled in the art would know how to produce the enzyme used in the present invention, there would be no undue experimentation needed to follow the teachings of the invention. The rejection of Claims 1-16 under 35 USC §112, first paragraph, should therefore be withdrawn.

Claims 1-16 have been rejected under 35 USC §112, second paragraph, as being indefinite for failing to point out and distinctly claim the subject matter of the invention. The Examiner believes that the claims are confusing and indefinite because the Examiner believes that Claim 1 is inconsistent with Claim 7. The Examiner believes that the terminology used in Claim 1 requires the elimination of ß-(1,6) linked chains,

whereas Claim 7 simultaneously requires "ß-(1,3) side chains attached by ß-(1,6) linkages and yet also requires glucan to be "essentially free of ß-(1,6) linked chains. The Examiner believes that the specification teaches that in order to encompass the preparation of products wherein ß-(1,6) linked chains having more than one consecutively ß-(1,6) linked glucose residues are eliminated from a yeast glucan product. The claims have been amended to describe the number of ß-(1,6) bound glucose units in order to provide more clarity to the claims. Thus, some chains are present, those having four or less ß-(1,6) bound glucose units. Support for this amendment is found on page 4, lines 14-16 of the specification.

Additionally, the Examiner objects to the use of the term "essentially free" in Claims 1, 7, 8, 9, 13 and 16 as being indefinite because it is not clear what percentage of ß-(1,6)-linked chains must be eliminated for a glucan to be considered essentially free of such chains. The amendment to the claims as described above obviates this rejection.

The Examiner has stated that Claim 2 is indefinite for reciting a single group, wherein the plural should have been used. Correction has been made to the claim.

The Examiner has further objected to the apparent indefiniteness of the use of the term "suitable" stating that it is subjective therefore indefinite. Applicants state that in the context, the use of the term "suitable" is definite and would be understood to one skilled in the art. Applicants are entitled to be their own lexicographers.

The Examiner has objected to the use of the phrases "characterized as" or "characterized by" In Claims 7-9 and 16. The claims have been amended to delete these phrases.

The Examiner is rejecting language in Claims 9, 10 and 11 which recite "especially from the yeast family *Saccharomyces*". The claim also recites "particularly from the yeast species *Saccharomyces cerevisiae*". The Examiner believes that the narrow limitation with the broader description of the yeast renders the claim indefinite. The claims have been amended and new dependent Claims 17-19 have been added to obviate this rejection.

The Examiner has objected to the spelling of "particularly". The term has been removed or correctly spelled in the claims. Applicant has not noted misspellings of the term in the specification.

In view of the foregoing comments and amendments to the claims, Applicants submit that the claims meet the requirements of 35 USC §112, second paragraph, and are definite. Applicants request that the rejection be withdrawn.

Claims 1, 4, 5, 7, 9, 10, 13, 14 and 16 have been rejected under 35 USC §102(b) as being anticipated by the Shiota et al. reference, J. Biochem. 98:1301-1307 (1985). The Examiner believes that the Shiota et al. reference discloses a process wherein *Saccharomyces cereviseae* beta-glucan is hydrolyzed by an endo-ß-(1,6) glucanase, therefore the reference anticipates the claimed processes and products. The ß-(1,6) glucanase used by the Shiota et al. reference is from a different microorganism than from the claimed ß-(1,6) glucanase. Further, the Shiota et al. discloses acetic acid as a solubilizing agent. This is not disclosed in the present application. Most importantly, the resulting beta-glucans described by the Shiota et al. reference are not the glucans described and claimed in the present application. The claimed invention relates to a glucan comprised of ß-(1,3)-linked glucose units and is essentially free of ß-(1,6)-linked chains apart from those chains of four or less ß-(1,6) bound glucose units.

Anticipation of a claim requires the disclosure of each and every recitation as set forth in the claims in a prior art reference. *Titanium Metals Corp. v. Banner, 227 USPQ 773 (Fed. Cir. 1985); OrthoKinetics, Inc. v. Safety Travel Chairs, Inc., 1 USPQ 2nd 1081(Fed. Cir. 1986); Akzo NV v. US International Trade Commissioner, 1 USPQ 2nd 1281(Fed. Cir. 1986).* There must be no difference between the claimed invention and the referenced disclosure for an anticipation rejection under 35 USC §102. *Scripts Clinic and Research Foundation v. GeneTech, Inc. 18 USPQ 2nd 1001 (Fed. Cir. 1991); Studiengeselschaf Kohle GnvH v. Dart Industries 220 USPQ 841(Fed. Cir. 1984).* In view of the foregoing authority, since the cited reference fails to disclose each and every recitation of the claims, the rejection should be withdrawn.

Claims 1-3 have been rejected under 35 USC §103(a) as being unpatentable over the Shiota et al. reference in view of the de la Cruz et al. reference. The Examiner further explains that Claims 2 and 3 limit the ß-(1,6) glucanase of Claim 1 to an enzyme

obtained from *Trichoderma harzianum*. The ß-(1,6)-glucanase used by Shiota is from a different microorganism than the claimed glucanase. The Examiner states that the De La Cruz reference discloses ß-(1,6) glucanase and therefore it would be obvious to use this glucanase in the Shiota et al. process. Neither the Shiota et al. reference or the de la Cruz reference is directed to a process for preparing the glucan of the present invention as it is currently claimed. Claim 1 relates to a process for preparing a glucan comprised of ß-(1,3)-linked glucose units as essentially free of ß-(1,6)-linked chains apart from those chains of four or less ß-(1, 6) bound glucose units. No matter what microorganism is used in the preparation of the glucanase, the resulting glucan product of the present invention is different from the product described in the Shiota et al. and de la Cruz references and therefore the substitution of a glucanase cannot render the claims 1-3 obvious. Therefore, the objection should be withdrawn.

Claims 1, 6, and 13-15 are rejected under 35 USC §103(a) as being unpatentable over the Shiota et al. reference in view of the Jamas reference, US Patent No. 5,028,703. The Examiner states that Claims 6 and 15 limit the processes of Claims 1 and 13 to processes wherein specific extraction steps are performed. The Examiner admits that the Shiota et al. reference does not disclose processes wherein the exact process steps recited in Claims 6 and 15 of the present application are performed. The Examiner further states that the Jamas et al. reference teaches that prior to the acid or enzymatic treatment, glucan derived from *Saccharomyces cereviseae* can be extracted from yeast using a variety of extraction techniques under a variety of conditions including those allegedly disclosed in present claims 6 and 15. Therefore one skilled in the art can substitute the disclosure of Jamas into the disclosure of Shiota et al. and arrive at the teachings of Claims 6 and 15 of the present invention. Applicants disagree with this rejection for various reasons, the most important being that the glucan product of the teachings of the Shiota et al. and Jamas references does not relate to the claimed glucan of the present invention. The glucan of the present invention comprises ß-(1,3)-linked glucose units and is essentially free of ß-(1,6)-linked chains apart from those chains of four or less ß-(1,6) glucose units. This difference alone would not lead one skilled in the art to the alleged combination of teachings from the Shiota et al. and

Jamas references to render obvious the claimed invention. Therefore, the rejection should be withdrawn.

Claims 10 and 11 have been rejected under 35 USC §103(a) as being unpatentable over the Shiota et al. reference, in view of the Jamas reference and further in view of the Matsueda et al. reference GB2 076 418. The Examiner states that Claim 11 of the present invention limits process Claim 10 to one in which formic acid is employed as the acid solubilizing agent. The Shiota et al. reference discloses acetic acid as a solubilizing agent. The Jamas et al. reference discloses acetic acid and the Matsueda reference discloses that formic acid can be used as a pre-enzymatic hydrolysis solubilizing agent for a specialized glucan having a ß-(1,3) glucan backbone and ß-(1,6) branch structures. The Examiner believes that one skilled in the art would recognize that the formic acid as used by Matsueda could be substituted for the acetic acid used in the Shiota reference given the teaching in the Jamas reference that acids mild enough to preserve the ß-(1,3) glucan backbone could have been used equivalently to acetic acid.

The enzymatic treatment of glucan particles do not solubilize the glucan particles described in the present application. The solubilization process uses formic acid as described in Claim 11, after the enzymatic treatment of the glucan particles. The solubilized glucans resulting from the enzymatic digestion as described in the Shiota et al. reference are solely glucose together with some amount of gentiobiose. Further, acetic acid will not act as a solubilizing agent on yeast beta glucan. There is no teaching in the Shiota et al. reference indicating that acetic acid has been used as a solubilizing agent for yeast beta-glucan. In the text of the reference at column 6, line 67 through column 7, line 3 it is stated that acetic acid will be able to hydrolyze preferentially ß-(1,6) linkages without affecting the ß-(1,3) linkages. There is no reference to "solubilizing". In order to solubilize the yeast beta glucan, a limited number of beta-(1,3) linkages is absolutely necessary. This can be done by the use of acids other than acetic if there is no teaching in the prior art that the branched structure of a solubilized glucan is maintained while retaining its solubility in aqueous solutions and its potential as an immuno-stimulatory agent.

The claimed process of the present invention treats particulate glucans in such a way that the branched structure is maintained and unwanted ß-(1,6) linked chains are removed, leaving a starting material suitable for formic acid hydrolysis, producing a highly solubilized product as described in Claims 10 and 11. The resulting product is characterized by its branched structure where the side chains are ß-(1,3) linked. The importance of the branched structure has been recited previously.

The Matsueda et al. reference describes a method for increasing the solubility of an already soluble beta glucan product having a ß-(1,3) linked backbone with single glucose units attached to the main chain for every 3.5 glucose units in the main chain. The reference describes a glucan with a side glucose groups rather than the side chains (chain referring to a certain number of interconnected glucosyl units) and differs significantly from the structure of the glucan claimed in the present application.

Claims 8 and 12 have been rejected under 35 USC §102(b) as being anticipated by, or in the alternative under 35 USC §103 as obvious over the Shiota et al. reference. The Examiner states that the Shiota et al. reference discloses a glucan product which appears to be identical to the presently claimed glucan product because the product results from contact in the claimed starting material, *Saccharomyces cereviseae* glucan, with the claimed enzyme, ß-(1,6) glucanase. The Examiner states that even if the glucan product of the Shiota reference is not anticipatory of the presently claimed invention, it would render the claimed glucan obvious to one of ordinary skill in the art. The Examiner makes no support for these comments other than quoting MPEP Section 706.3(e). The claimed product of the present invention is not the product disclosed by Shiota et al. nor by the Shiota et al. reference in combination with one or more of the other prior art references. None of the references discloses a process for producing glucan or a glucan product comprised of ß-(1,3) linked glucose units that is essentially free of ß-(1,6) linked chains apart from those chains of four or less ß-(1,6) bound glucose units. At least for this reason, the prior art reference does not teach or suggest the claimed invention. It is noted that to establish a *prima facie* case of obviousness under §103, all claim limitations of the claimed invention must be taught or suggested by the prior art. *In re Royka 490 F.2d 981, 180 USPQ 580 (CCPA 1974).* In view of the foregoing authority and since the references do not teach or suggest a "resulting glucan

comprised of ß-(1,3) linked glucose units that is essentially free of ß-(1,6)-linked chains apart from those chains of four or less ß-(1,6) bound glucose units. as claimed. The cited references fail to support the asserted rejections under 35 USC §103. Withdrawal of the rejections of the claims is required.

In view of the foregoing comments and amendments to the claims, Applicants submit the claims meet the requirements of 35 United States Code. An early Notice of Allowance is respectfully requested.

Respectfully submitted,

May 2, 2003
Date

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DOCKET: CU-1446

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

APPLICANT:	Rolf Engstad et al.	)	
		)	
SERIAL NO:	08/716,344	)	Group Art Unit: 1808
		)	
FILING DATE:	October 2, 1996	)	Examiner: F. Prats
		)	
TITLE:	Enzyme Treatment of Glucans		

The Assistant Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

MARKED VERSION OF AMENDED CLAIMS

1. A process for the preparation of a glucan product from yeast which comprises:
[a)] contacting a particulate branched ß-1,3-glucan having ß-1,3-linked and ß-1,6-linked chains therein with a ß-1,6-glucanase under conditions such that the resulting glucan is comprised of ß-1,3-linked glucose units and is essentially free of ß-1,6-linked chains**, containing four or less ß-1,6-bound glucose units**.

A2

2. A process according to Claim 1 wherein said ß-1,6-glucanase is obtained from the **[groups] group** of microorganisms consisting of *Trichoderma longibrachiatum*, *Trichoderma reesei*, *Trichoderma harzianum*, *Rhizopus chinensis*, *Gibberella fujikuroi*, *Bacillus circulans*, *Mucor lilmalis* and *Acinetobacter*.

7. The product of the process of Claim 1, being **[characterized as] being insoluble and particulate having** a particulate branched ß-1,3-glucan with ß-1,3-linked side chains being attached by a ß-1,6-linkage and being essentially free of ß-1,6-linked chains**, containing four or less ß-1,6-bound glucose units**.

A3

8. The product of the process of Claim 6, being **[characterized as] an insoluble, particulate** a branched ß-1,3-glucan with ß-1,3-linked side chains being attached by a ß-1,6-linkage and being essentially free of ß-1,6-linked chains**, containing four or less ß-1,6-bound glucose units**.

9. An insoluble particulate yeast glucan especially from the yeast family *Saccharomyces* **[and particularly from the yeast species *Saccharomyces cerevisiae* being characterized as] wherein** a branched ß-1,3-glucan with ß-1,3-linked side chains being attached by a ß-1,6-linkage and being essentially free of ß-1,6-linked chains**, and containing four or less ß-1,6-bound glucose units**.

10. A process for the production of a solubilized ß-(1-3)-glucan particle from yeast, **[especially from yeast family *Saccharomyces* and particularly from the yeast species *Saccharomyces cereviseae*]** which comprises contacting an insoluble glucan from yeast **[family *Saccharomyces*]** having a backbone of

A3 cont.

ß-(1-3)-linked glucose units with at least one ß-(1-3)-linked side chain of at least 1 glucose units attached thereto with a solubilizing agent.

13. A process for the preparation of a feed glucan product from yeast, **[especially from the yeast family *Saccharomyces* and particularly from the yeast species *Saccharomyces cerevisiae*,]** which comprises:

A4

[a)] contacting the feed grade yeast glucan being a branched ß-1,3-glucan having ß-1,3-linked and ß-1,6-linked chains therein with a ß-1,6-glucanase under conditions such that the resulting glucan is comprised of ß-1,3-linked glucose units and is essentially free of ß-1,6-linked chains **, containing four or less ß-1,6-bound glucose units**.

A5

16. The product of the process of Claim 13, **[being characterized as]** **comprising** a branched ß-1,3-glucan with ß-1,3-linked side chains being attached by a ß-1,6-linkage and being essentially free of ß-1,6-linked chains **, containing four or less ß-1,6-bound glucose units**.